AD_____

Award Number: W81XWH-04-1-0277

TITLE: Quantitative in Situ Assessment of the Somatostatin
        Receptor in Breast Cancer to Assess Response to Targeted
        Therapy with 111-In-Pentetreotide

PRINCIPAL INVESTIGATOR: Gina G. Chung, M.D.
                         John Murren, M.D.
                         David Rimm, M.D., Ph.D.

CONTRACTING ORGANIZATION: Yale University School of Medicine
                           New Haven, Connecticut 06520-8047

REPORT DATE: May 2005

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
               Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
                        Distribution Unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE May 2005	3. REPORT TYPE AND DATES COVERED Annual (1 Apr 2004 – 31 Mar 2005)

4. TITLE AND SUBTITLE
Quantitative in Situ Assessment of the Somatostatin Receptor in Breast Cancer to Assess Response to Targeted Therapy with 111-In-Pentetreotide

5. FUNDING NUMBERS
W81XWH-04-1-0277

6. AUTHOR(S)
Gina G. Chung, M.D.
John Murren, M.D.
David Rimm, M.D., Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Yale University School of Medicine
New Haven, Connecticut 06520-8047

E-Mail: Gina.chung@yale.edu

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES
Original contains color plates: All DTIC reproductions will be in black and white.

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 Words)

Somatostatin (SST) is a peptide hormone implicated in the growth and progression of cancers and SSTR2 is the predominant receptor subtype expressed in breast cancer. We hope to study the feasibility and efficacy of radiolabelled somatostatin analogs in breast cancer. To this end, we have developed an algorithm called AQUA that can assess protein expression on tissue microarrays (TMA) based on molecular co-localization techniques. Our results show that SSTR2 is variably expressed in a large proportion of breast cancers and is predominantly within the membrane compartment of tumors not stroma. Although expression was not significantly correlated with survival on our TMA, it did appear to be overexpressed in malignant breast epithelium compared with benign breast tissue. We will quantitate protein measurement even further with correlations with cultured cell line expression of SSTR2. We have also begun efforts to incorporate our techniques clinically by opening a clinical trial in patients with breast cancer to analyze our methodologies for SSTR2 expression with more traditional methods. Ultimately, our plan is to compare the different measures of SSTR2 expression on predicting response/survival in a clinical trial of radiolabelled SST analogue (111-In-pentetreotide) in patients with metastatic breast cancer.

14. SUBJECT TERMS
Breast cancer, quantitative analysis, tissue micrarray, somatostatin receptor, radiolabelled somatostatin analogue, clinical trial

15. NUMBER OF PAGES
23

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT Unlimited

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)
Prescribed by ANSI Std. Z39-18
298-102

Table of Contents

Introduction

Somatostatin (SST) is a peptide hormone that inhibits the release of various hormones and growth factors. The receptors are also expressed in numerous tumors, with SSTR2, the predominant subtype expressed in breast cancer. Although there are data for inhibitory effects of SST analogues in breast cancer, the feasibility and/or efficacy of radioisotope-conjugated somatostatin analogs in breast cancer (studied more extensively in neuroendocrine tumors) are unknown and SST_R status prior to treatment were minimally investigated and varied in these studies. Until recently, SST_R expression has been performed by labor intensive methods such as autoradiography and RT-PCR *in vitro* and scintigraphy *in vivo*. We have developed a series of algorithms called AQUA that can assess protein expression on tissue microarrays (TMA) based on molecular co-localization techniques. Our automated analyses involves immunohistochemistry (IHC) combined with automated acquisition and analysis of compartmentalized, quantitative, continuous scores which removes the inherent subjectivity of standard pathologist-based scoring systems. We propose to further characterize the expression of SST_R2 using a large cohort breast cancer TMA and to correlate *in situ* tissue measurements by AQUA with that of scintigraphy and RT-PCR. Ultimately, our plan is to compare the different measures of SSTR2 expression on predicting response/survival in a clinical trial of radiolabelled SST analogue (111-In-pentetreotide) in patients with metastatic breast cancer.

Body

Task 1. Characterize SSTR expression in a breast cancer TMA

Because our initial breast cancer TMA was nearly exhausted, we constructed a 2nd fold redundant TMA with the same cases. A 3rd fold redundant array is also currently being constructed to allow experiments to be reproduced and to gather 2 fold redundant data. We have collected extensive clinical and pathologic data on these patients, including disease-specific and overall survival. SSTR2 antibody from Santa Cruz, Carpinteria, CA) was obtained and initially titered on a breast cancer test array (TMA but with much fewer spots and without linkage to clinical information) to determine an optimal dilution. The full cohort TMA with 667 cases was then stained with SSTR2 and analyzed with AQUA (Figure 1). SSTR2 stained predominantly in the invasive tumors in a membranous pattern. This is consistent with other reports of SSTR2 expression in cancers (1-3). There also appeared to a lesser extent, variable levels in the stroma and vascular/lymphatic structures as well.
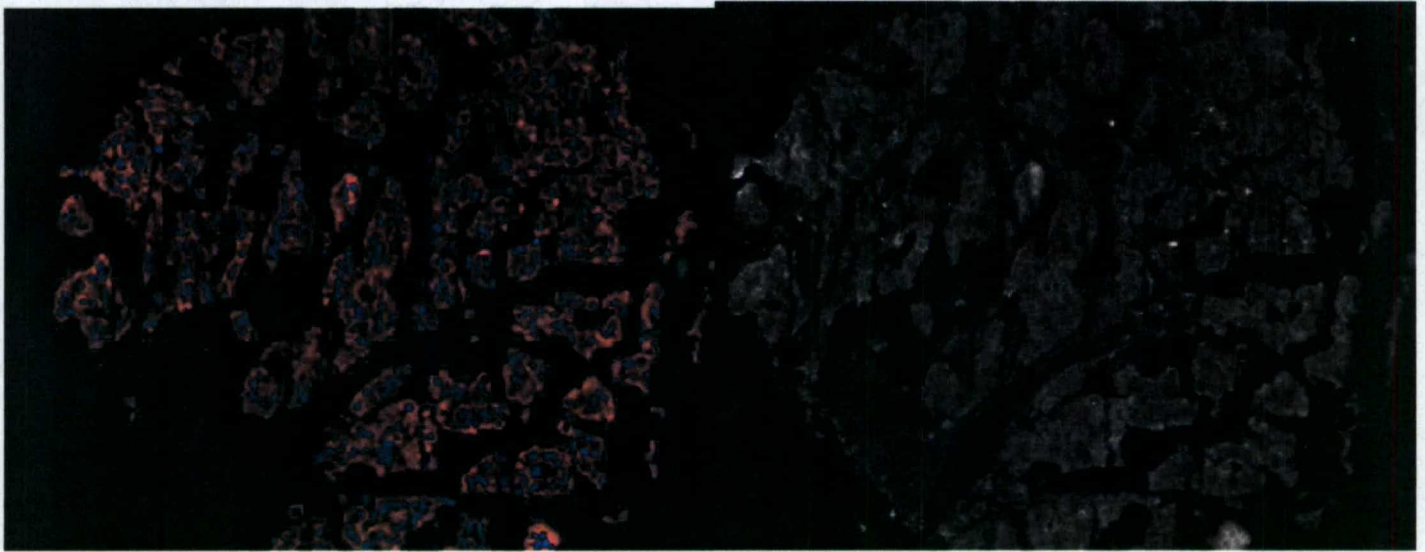
Figure 1. Representative histospot of breast cancer stained with SSTR2 (right panel and composite image (left panel). Red represents SSTR2 colocalized with membrane and blue is SSTR2 colocalized to nucleus.

Outcome analysis showed that the standard markers such as tumor size, nodal status, and estrogen receptor, but not SSTR2 expression in the tumors were associated with disease-specific survival. Using X-tile (a statistical model developed for determination of optimal cutpoints of expression), the highest expressers were significantly associated with markers of poor prognosis (e.g. positive nodal status, large tumor size). Please see Appendix A.

In order to better compare SSTR2 expression in breast cancers versus normal breast epithelium, we also constructed and analyzed a TMA of normal breast tissue. These specimens were obtained predominantly from patients undergoing reduction mammoplasties. This showed again that SSTR2 stained predominantly within the membrane compartment of the epithelium but that the expression as a whole was substantially diminished compared to our breast cancer cohort (Figure 2).
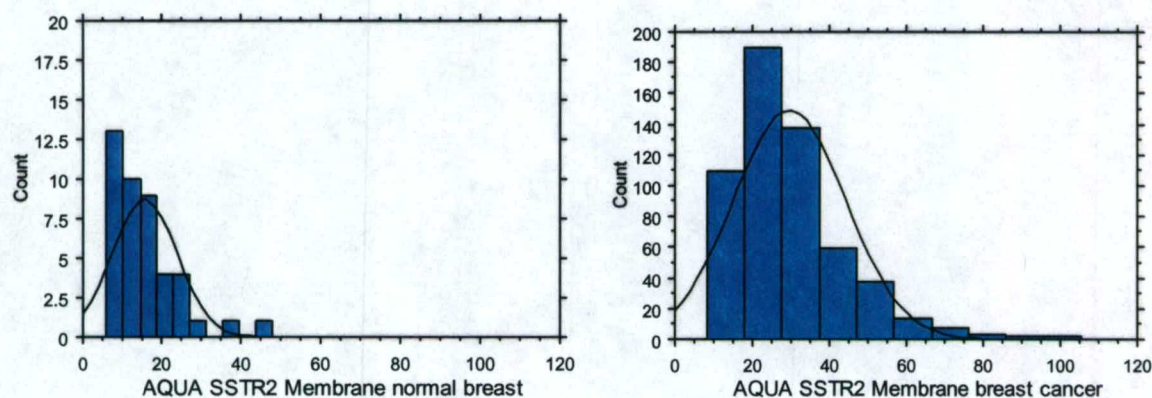
Figure 2.
SSTR2 scores in normal and malignant breast TMAs. Mean of normal scores is 15 and malignant is 29.

Our results will be reproduced on our 3rd fold redundant array currently being constructed, however, the clear overexpression of SSTR2 in tumors and the predominant tumoral rather than stromal localization is encouraging given our future plans to use SSTR2 as a homing target for radiolabelled somatostatin analogues in advanced breast cancer.

Finally, in a collaboration with Dr. John Murren (a coinvestigator on this grant), we have analyzed SSTR2 expression in a sarcoma TMA and a multi-tumor TMA. These results are still being analyzed. It is the hope that these results will help us to obtain a more global sense of SSTR2 expression in many different tumors (as well as in their normal counterparts) so that further investigations into the biology of this pathway in tumors can be initiated and that therapeutic studies can be initiated in select malignancies. Initial work comparing immunohistochemical analysis of SSTR2 expression with RT-PCR and scintigraphy on a series of sarcoma samples is currently being prepared as a manuscript for submission.

Task 2. Translating TMA-based AQUA algorithms to whole sections

We have been doing initial studies for this task with estrogen receptor (ER) on whole tissue sections because this is a well characterized marker in breast cancer in which a pathologist-based "gold standard" exists. Multiple slides of breast cancer from the same patient were obtained from different blocks. These data analysis are still ongoing, but initial results suggest that the intra and inter slide variabilities per case were quite low for the low ER (ER negative cases) whereas the higher ER score cases had more variability. Figure 3 shows "heat maps" of AQUA ER scores on different slides with green representing scores close to the mean and red representing scores further away from the mean. In the high scoring case (e.g. S003063), there is a suggestion that highest scores are clumped together. These data will be further analyzed and also compared with the pathologist's scores.
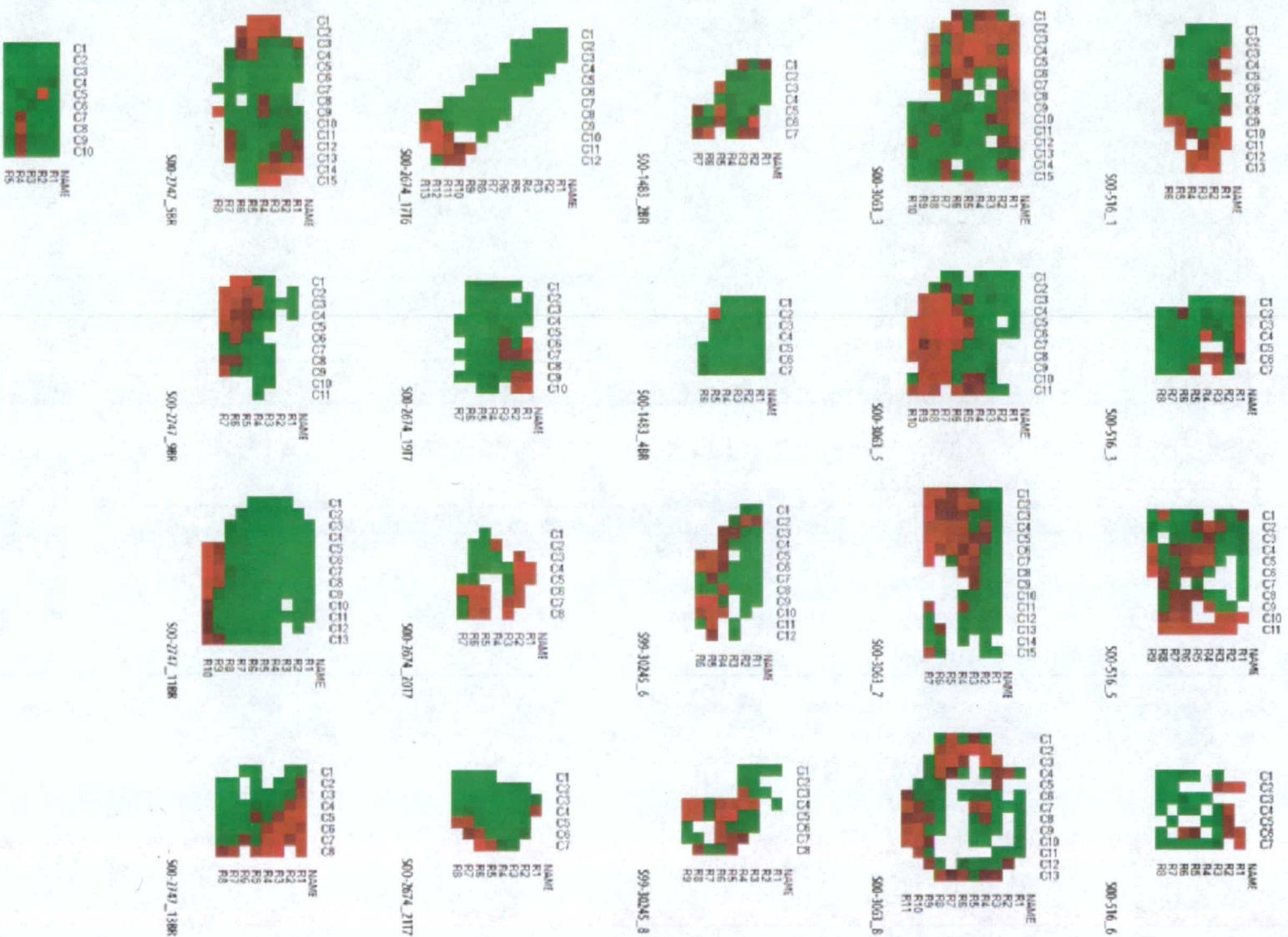
Figure 3

Task 3. Conversion of AQUA to a protein concentration

We have gathered a series of cultured cell lines from ATCC for this task. These include breast cancer lines (MB175UN, BT474, SKBR3, MB468, MCF7) and other malignancies as well (A431, SW480). Although all these lines have not previously been analyzed, MCF7 did show a strong signal for SSTR2 by RT-PCR. These lines were processed into a cell line microarray using a technique involving fixation, resuspension and pelleting only, and then paraffin embedding. This redundant cell line array is then added to the same slide as the breast cancer TMA for staining with SSTR2 and AQUA analysis concomitantly with the malignant tissue specimens. The AQUA scores for these cell lines were quite variable and had a fairly wide range (nearly the range of AQUA scores seen in the tumor specimens on the TMA) (Figure 4).
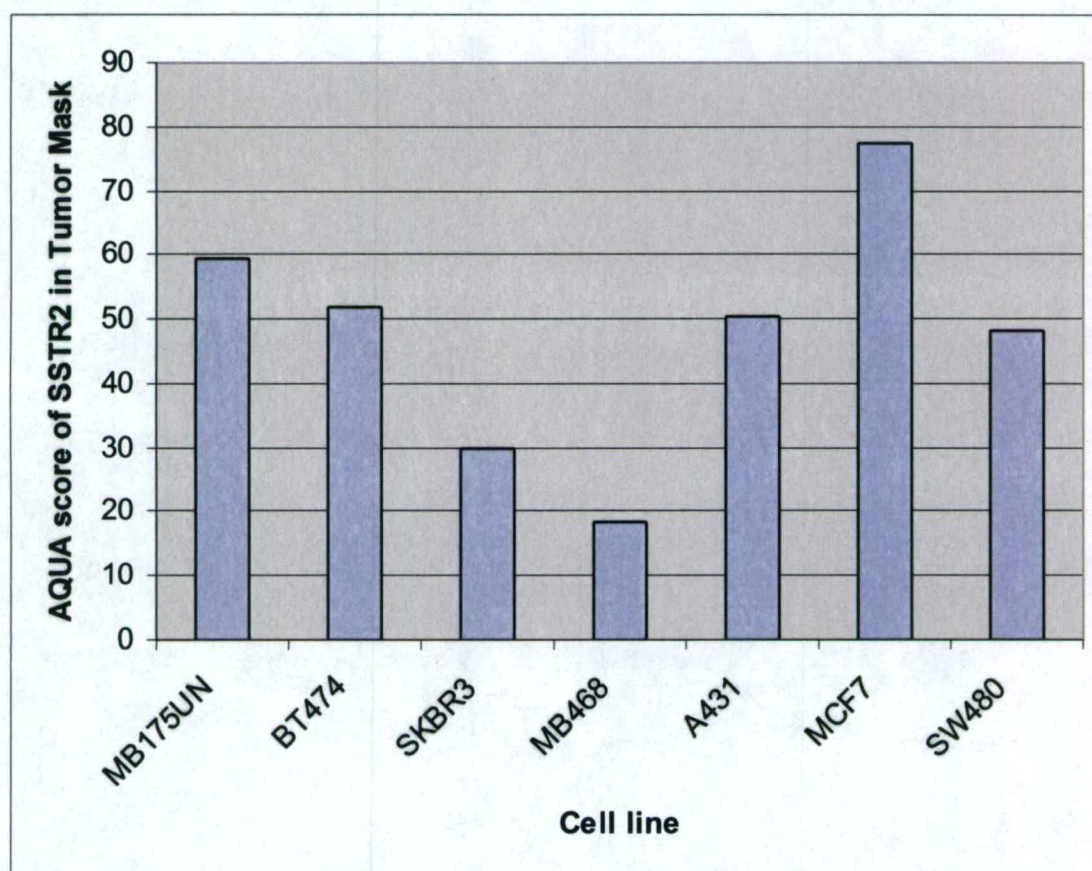

Figure 4.

As expected, MCF7 had high tumor mask and membrane expression. Additional cell lines will be tested to increase the dynamic range. All cell lines will be analyzed by ELISA for a quantitative measure of SSTR2 to correlate with the AQUA scores.

Task 4. Study 111-In-pentetreotide activity and safety in patients with metastatic breast cancer

Prior to a prospective clinical trial assessing the therapeutic efficacy of 111-In-pentetreotide, we are again collaborating with Dr. Murren in an ongoing trial of patients with sarcomas. This study recruits patients with sarcomas having definitive resections at Yale New Haven Hospital and simply compares SSTR2 expression by three different methodologies, RT-PCR on resected tissue, immunohistochemistry on resected specimen, and pre-surgery scintigraphy with ^{111}In-pentetreotide (Octreoscan). Eight patients have been enrolled to date and no serious or unanticipated adverse events have been seen. Data analysis from this study is ongoing. This protocol is now being amended to include patients with breast cancer having surgical biopsy/resection at Yale New Haven Hospital (See appendix B for amended protocol). Patients may be of any stage, although scintigraphy must be prior to resection/biopsy and both procedures performed preferably prior to any other therapeutic treatments. The amended study is currently being evaluated by the Human Investigations Committee at Yale.

Key Research Accomplishments
1. Construction of a 2nd and third fold redundant breast TMA
2. Titrations of SSTR2 antibody on test arrays
3. Correlation of SSTR2 expression on a large cohort breast cancer TMA with clinico/pathologic parameters
4. Construction of normal breast TMA and testing SSTR2 on normal breast TMA
5. Optimizing AQUA analysis on whole tissue sections with estrogen receptor as a model
6. Preliminary work on validating SSTR2 antibodies on cultured cell lines and conversion of AQUA scores to protein concentrations
7. Clinical protocol studying SSTR2 expression by three different methodologies in patients with sarcomas or breast cancers

Reportable Outcomes
1. DOD Era of Hope Breast Cancer Research Meeting June 2005: QUANTITATIVE ANALYSIS OF SOMATOSTATIN RECEPTOR-2 ON A BREAST CANCER TISSUE MICROARRAY
2. Yale University HIC protocol 12513: Characterization of Somatostatin Receptor Expression in Sarcomas and Breast Cancer

Conclusions

We have begun a systematic analysis of the expression of the SSTR2 in breast cancer using our automated analysis methodology which allows rapid, reproducible, quantitative measurements of in situ protein expression on tissue arrays. Our results show that SSTR2 is expressed in a graded fashion in a large proportion of breast cancers, is expressed predominantly within tumors not stroma, and that it is mostly expressed in the membrane compartment of tumors. Although expression was not significantly correlated with survival on our TMA, it did appear to be significantly overexpressed in malignant breast epithelium compared with benign breast tissue. We will quantitate protein measurement even further with correlations with cultured cell line expression of SSTR2. We have also begun efforts to incorporate our techniques and results in the clinical setting by opening a clinical trial in patients with breast cancer to analyze our methodologies for SSTR2 expression with more traditional methods. We hope to eventually open a clinical trial of radiolabelled octreotide as a therapeutic maneuver for patients with refractory metastatic breast cancer and further study the different methods of SSTR2 expression specifically as a predictive factor to targeted therapy.

References

1. Janson ET, Stridsberg M, Gobl A, et al. 1998 Determination of somatostatin receptor subtype 2 in carcinoid tumors by immunohistochemical investigation with somatostatin receptor subtype 2 antibodies. Cancer Res 58:2375-2378
2. Kimura N, Pilichowska M, Date F, et al. 1999 Immunohistochemical expression of somatostatin type 2A receptor in neuroendocrine tumors. Clin Cancer Res 5:3483-3487
3. Reubi JC, Kappeler A, Waser B, et al. 1998 Immunohistochemical localization of somatostatin receptors sst2A in human tumors. Am J Pathol 153:233-245

Appendices

A. QUANTITATIVE ANALYSIS OF SOMATOSTATIN RECEPTOR-2 ON A BREAST CANCER TISSUE MICROARRAY

Yale Cancer Center, Medical Oncology and Department of Pathology, Yale
University School of Medicine, New Haven, CT 01520
Gina G. Chung, Sriparna Ghosh, Maciej P. Zerkowski, Robert L. Camp, David
L. Rimm, John Murren
gina.chung@yale.edu

Background: Somatostatin is a peptide hormone that inhibits the release of
various hormones and growth factors. The receptors are also expressed in
numerous tumors, with SSTR2, the predominant subtype expressed in breast
cancer, implicated in its growth and development. Using a large cohort
tissue microarray (TMA) of node negative and positive breast cancers, we
analyzed the protein expression and subcellular localization of SSTR2 by
our automated, quantitative in situ analysis of immunostains (AQUA) and a
newly developed statistical analysis for determination of optimal
cutpoints of expression (X-tile).

Methods: Our TMA consisted of 667 cases of primary breast cancers, equally
divided between node negative and positive cancers. AQUA uses a modified
immunohistochemistry technique with fluorochromes as the detection method.
Primary antibody was SSTR2 (Santa Cruz, Carpinteria, CA). The total tumor
and subcellular distribution pattern of SSTR2 were then correlated with
clinicopathologic factors and with survival. Survival curves and cutpoint
analysis were determined with X-tile, our recently developed software for
determination of optimal cutpoints on continuous data. Chi-square and
multivariate analysis were performed with Statview software (version
5.0.1; SAS Institute Inc., Cary, NC).

Results: SSTR2 stained more strongly in tumors than benign controls and
predominantly in the tumor membrane, although some stromal staining was
also seen. Peritumoral vessel staining could not be deliniated in our
experiment and some expression may have been included within the "tumor
mask" by AQUA. X-tile divided expression into the top 17% expressers
versus the remaining 83%. Kaplan Meier survival curves showed that
whereas the high expressers trended towards worse 20 year disease-specific
survival compared with the low, this did not reach statistical
significance (Corrected P-value=0.2750). Nuclear staining was
occasionally seen, however it was not prognostic. Univariate analysis of
standard prognostic markers showed that in our cohort, tumor size, nodal
status, nuclear grade, estrogen and progesterone receptor status, but not
Her2/neu were all significantly correlated with outcome. Using X-tile,
the Cox continuous variable of SSTR2 membrane expression was also

correlated with outcome (p= 0.0460). Chi-square analysis using the X-tile determined SSTR2 optimum cut-point of 40.7 showed that high SSTR2 expression was significantly associated with positive nodal status, larger tumors, and high nuclear grade.

Conclusions: These results indicate that SSTR2 may be variably overexpressed in breast cancer, is predominantly expressed within tumor membranes, and that it is associated with other markers of poor prognosis. High expression showed a non-significant trend towards worse disease-specific survival. Furthermore, AQUA and X-tile analyses suggest an optimal cutpoint for future investigations of this biomarker and specifically, in studies looking at its expression as a predictive biomarker in therapeutic studies of radioactive targeting agents against SSTR2 in breast cancer.

B. Characterization of Somatostatin Receptor Expression in Sarcomas and Breast Cancer

<u>TABLE OF CONTENTS</u>

A. PURPOSE

1. Characterize the expression of somatostatin type 2 receptors (SST_R2) in sarcomatous lesions and in breast cancers.
2. Compare three methods for assaying expression of SST_R2
   a. Uptake of ^{111}In-pentetreotide by scintigraphy (Octreoscan)
   b. RT-PCR (reverse transcription polymerase chain reaction)
   c. Immunocytochemistry

B. BACKGROUND

Current treatment options for sarcomas include wide surgical resection, radiation therapy, and chemotherapy [1-3]. Different combinations of these treatment regimens are used depending on the type of sarcoma and extent of disease. Some sarcomas are not sensitive to current methods of chemotherapy and, as a group, sarcomas have a 5-year survival rate of 60-75% [3,4]. Novel chemotherapeutic approaches are needed and research in this field is ongoing.

Breast cancer is the second leading cause of cancer deaths in women in the U.S. [5]. Despite advances in treatment, nearly all patients with metastatic breast cancer and a significant proportion of patients with early stage disease will also relapse and die of their disease emphasizing the need for more effective drugs [6].

Octreotide is a synthetic octapeptide analog of somatostatin (SST), a peptide hormone with predominantly inhibitory effects on the secretion of growth factors and exocrine hormones as well as on cellular proliferation. The actions of somatostatin and analogs are mediated by somatostatin receptors, which are members of the G-protein family of receptors [7,8]. Five distinct somatostatin receptor subtypes have been identified, and the respective genes have been cloned [8-12]. Of these receptors, somatostatin receptor 2 (SST_R2) appears to be strongly expressed most commonly in human tumors. Octreotide binds with high affinity to SST_R2, with low affinity to SST_R3 and SST_R5, whereas it does not bind to SST_R1 and SST_R4 [12-14]. These binding characteristics led to the development of radiolabeled octreotide as an imaging agent for tumors expressing SST_R2. Somatostatin receptor imaging with [^{111}In-DTPA-D-Phe]-octreotide (^{111}In-pentetreotide or Octreoscan), a [111]Indium-labelled octreotide with DTPA as a chelating agent [15], has been approved by the FDA for patients with tumors with somatostatin receptors.

A variety of human tumors, both endocrine and non-endocrine, express somatostatin receptors [16]. Endocrine gastroenteropancreatic tumors have been studied most extensively [17,18]. Findings of in vivo imaging with radiolabeled octreotide and in vitro autoradiographic analyses of somatostatin receptors in these types of tumors have been shown to correlate well when directly compared [19].

It has been demonstrated through in vitro studies that somatostatin receptors are present in human mesenchymal tumors [20,21]. In vivo scintigraphic studies have demonstrated uptake of radiolabelled somatostatin analog in 71-92% of patients with sarcomas [22-24]. Animal studies have demonstrated the effectiveness of somatostatin analogs to inhibit the growth and/or angiogenesis of certain sarcoma models [25-29].

SST_R2 is the predominant subtype expressed in breast cancers [30]. Because the synthetic SST analog octreotide has high affinity to this receptor, it is a rationale agent to study in this cancer. Indeed, several early studies of SST analogs in breast cancers have shown encouraging results [31].

It is not known which bone and soft-tissue sarcomas express SST_2R in highest quantities and would intuitively be most sensitive to treatment with a somatostatin analog either alone or labeled with a beta emitting isotope. There have also been no studies to see if SST_2R expression in sarcomas correlates with octreotide scanning. Similarly, in breast cancers, the expression pattern and distribution of SST_R2 in tumors have not been well characterized nor correlated with other measures of expression.

C.   **LOCATION OF STUDY**

The Oncology, Breast Surgery, and Orthopaedic Outpatient Clinics, the Diagnostic Imaging Center and the Operating Room of Yale-New Haven Hospital

D.   **PROBABLE DURATION OF PROJECT**

36 months

E.   **RESEARCH PLAN**

We will use three approaches to characterize the expression of SST receptors in patients with sarcomas and breast cancers.

1. Eligibility Criteria: Patients identified to have sarcomatous lesions or invasive breast carcinomas and who will have definitive resection at Yale-New Haven Hospital will be eligible for inclusion in this study.

2. Exclusion Criteria: Pregnant women

3. Octreoscans: Prior to tumor resection each patient will have an Octreotide scan to evaluate the intensity of radioisotope uptake within the primary tumor and/or metastatic foci.

   a. Preparation: None; Patients will be instructed to be well hydrated the morning of the injection.
   b. Radiopharmaceutical: 6 mCi of ^{111}In-pentetreotide will be injected intravenously into the arm. Following injection, the isotope is rapidly cleared from the blood (decrease within 10 minutes to 33% +/- 7%) and within 24 hours 85% of the injected dose is excreted through the kidney.
   c. Scanning protocol: Whole body and planar images are obtained at 4 hours post-injection. Patients will be asked to lie on their back on the examining table, and to stay as still as possible. Initial planar and whole body imaging will take approximately 1 hour. Patients will then leave with instructions to return the following day for planar and SPECT imaging at 24 hours post-injection. Imaging will require 1 hour.
   d. Dr. David Cheng will lead the interpretation of the octeoscans. ^{111}In-pentetreotide uptake within each area of tumor (analyzed in conjuction with CT/MRI) will be graded according to a three point scale: 0=no uptake, 1+=uptake less than liver, 2+=uptake greater than liver. Krenning et. al have proposed that the likelihood of response in therapeutic protocols is correlated with uptake that is 2+ on this scale[32].

4. Tissue analysis: At the time of definitive resection a cryostat specimen will be obtained from the operating room at Yale-New Haven Hospital and immediately transported to a -70°C freezer for storage. The remainder of the tissue will be processed in the routine manner (e.g. fixed and paraffin embedded). These tissues will subsequently be evaluated by reverse transcription PCR to determine the profile of somatostatin receptor subtypes (primarily SST_R2), and will be immunostained using a polyclonal antibody to determine the distribution (heterogeneity) of somatostatin type 2 receptor within the tumor.

F.   STATISTICAL CONSIDERATIONS

Our goal is to evaluate at least 20 patients with extremity sarcomas and 20 with breast carcinomas. We will see if there is any correlation between grade of scintigraphic uptake and somatostatin receptor expression as measured by RT-PCR and/or imunohistochemistry.

G. ECONOMIC CONSIDERATIONS

No material inducements or payments will be offered to subjects involved in this study. The Octreoscan is an FDA approved diagnostic study for patients with somatostatin receptor positive tumors, and the expense of imaging will be billed to the patient's insurance carrier.

H. HUMAN SUBJECTS

1. Subject Population

All patients identified by clinical evaluation, radiographic imaging or tissue biopsy to have a sarcomatous tumor or breast carcinoma are eligible for inclusion. There are no exclusion criteria with regard to age, gender, or ethnic background.

2. Source of Research Material

We plan to make use of existing pathology specimens that are obtained at biopsy procedures or definitive tumor resections.

3. Consent Procedures

Written informed consent will be obtained prior to definitive surgical resection after detailed discussion of the protocol in the outpatient Orthopaedic and/or Oncology clinic by one or more of the investigators identified on the face sheet.

4. Potential Risks

OctreoScan is an FDA approved imaging test that involves minimal risk to the subject. **The following side effects have been observed in less than one percent of 538 subjects receiving OctreoScan: dizziness, fever, flush, headache, a drop in blood pressure, changes in liver function, joint pain, nausea, sweating, and weakness. These side effects do not last long and are not permanent.**

Since ^{111}In-pentetreotide is derived from Octreotide, there is a remote chance that some subjects may experience pain at the OctreoScan injection site, diarrhea and/or loose stools, stomach pain, vomiting, a drop or increase in blood glucose levels, and/or hypertension. The scheduled OctreoScan dose is below the limits set by the FDA for research subjects.
The inconvenience of the scan consists of 1-2 hours of imaging time.

5. **Protection of Subject**
    Patient confidentiality will be strictly maintained in concordance with NIH guidelines.

6. **Potential Benefits**
    No direct benefit is expected to patients participating in this study.

7. **The Risk-Benefit Ratio**
    Knowledge gained from this study may lead to investigational protocols designed to assess the effectiveness of somatostatin analog therapy in patients with sarcomas and breast cancers.

8. **Data Safety and Monitoring Plan**
    One of the principle investigators will conduct a data and safety review on a quarterly basis. Serious unanticipated adverse events will be reported immediately to the HIC (using HIC form 6A) and any appropriate regulatory agencies. Other adverse events will be reported to the HIC periodically or, at minimum, when reapproval of the protocol is sought. The adverse event will be graded as serious or non-serious, and when possible determine if it is attributed to the research or not.

    Due to the nature of the research subjects' underlying diseases, they will be evaluated at frequent intervals by their treating physicians. Following the Octreoscan the subject will have a definitive resection of their tumor and will have close follow-up in the initial post operative period. These frequent interactions will allow for close monitoring of any adverse effects from the Octreoscan.

I. REFERENCES

1. Abeloff MD. Clinical Oncology, 2nd Edition. New York: Churchill Livingston 2000.
2. Enneking WF. Musculoskeletal tumor surgery. New York: Churchill Livingston, 1983.
3. Simon, M. Surgery for bone and soft-tissue tumors. Philadelphia: Lippincott-Raven, 1998.
4. Rougraff, BT. Survival rates for patients with musculoskeletal sarcomas. Current Opinion in Orthopaedics 1996;7:69-70.
5. National Center for Health Statistics. SEER cancer statistics review, 1973-1995. Bethesda, MD: US National Cancer Institute, 1998.
6. Harris JR, Lippma ME, Morrow M, Osborne CK. Diseases of the Breast (2nd edition), Lippincott Williams and Wilkins, 2000.

7. Pan MG, lorio T, Stork PJ. G protein activation of a hormone-stimulated phosphatase in human tumor cells. Science 1992; 256:1215-7.

8. Patel YC, Greenwood MT, Panetta R, Demchyshyn L, Niznik H, Srikant CB. The somatostatin receptor family. Life Sci 1995; 57:1249-65.

9. Pollack MN, Schally AV. Mechanisms of antineoplastic action of somatostatin analogs. Proc Soc Exp Biol Med 1998; 217:143-52.

10. Yamada Y, Reisine T, Law SF et al. Somatostatin receptors, an expanding gene family: cloning and functional characterization of human SSTR3, a protein coupled to adenylyl cyclase. Mol Endocrinol 1992; 6:2136-42.

11. Yamada Y, Post SR, Wang K, Tager HS, Bell GI, Seino S. Cloning and functional characterization of a family of human and mouse somatostatin receptors expressed in brain, gastrointestinal tract, and kidney. Proc Natl Acad Sci USA 1992; 89:251-5.

12. Yamada Y, Kagimoto S, Kubota A, et al. Cloning, functional expression and pharmacological characterization of a fourth (hSSTR4) and a fifth (hSSTR5) human somatostatin receptor subtype. Biochem Biophys Res Commun 1993; 195:844-52.

13. Buscail L, Delesque N, Esteve JP, et al. Stimulation of tyrosine phosphatase and inhibition of cell proliferation by somatostatin analogues: mediation by human somatostatin receptor subtypes SSTR1 And SSTR2. Proc Natl Acad Sci USA 1994;91:235-9.

14. Raynor K, Murphy WA, Coy DH, et al. Cloned somatostatin receptors: identification of subtype-selective peptides and demonstration of high affinity binding of linear peptides. Mol Pharmacol 1993; 43:838-44.

15. Bakker WH, Albert R, Bruns C, et al. [111In-DTPA-D-Phe1]-octreotide, a potential radiopharmaceutical for imaging of somatostatin receptor-positive tumors: synthesis, radiolabeling and in vitro validation. Life Sci 1991; 49:1583-91.

16. Reubi JC, Laissue J, Krenning E, Lamberts SW. Somatostatin receptors in human cancers: incidence, characteristics, functional correlates and clinical implication. J Steroid Biochem Mol Biol 1992; 43:27-35.

17. Kwekkeboom DJ, Krenning EP, Bakker WH, Oei HY, Kooji PP, Lamberts SW. Somatostatin analogue scintigraphy in carcinoid tumors. Eur J Nucl Med 1993; 20:283-92.

18. Jamar F, Fiasse R, Leners N, Pauwels S. Somatostatin receptor imaging with indium-111-pentetreotide in gastroenteropancreatic neuroendocrine tumors: safety, efficacy and impact on patient management. J Nucl Med 1995;36:542-9.

19. Lamberts SW, Hofland LJ, van Koetsveld PM, et al. Parallel in vivo and in vitro detection of functional somatostatin receptors in human endocrine pancreatic tumors: consequences with regard to

diagnosis, localization, and therapy. J Clin Endocrinol Metab 1990; 71:566-74.

20. Reubi JC, Waser B, Laissue JA. Somatostatin and vasoactive intestinal peptide receptors in human mesenchymal tumors: In vitro identification. Can Res 1996;56:1922-31.

21. Denzler B, Reubi JC. Expression of somatostatin receptors in peritumoral veins of human tumors. Cancer 1999;85:189-198.

22. Friedberg JW, Van der Abbeele AD, Kehoe K, Singer S, Fletcher CD, Demetri GD. Uptake of radiolabelled somatostatin analog is detectable in patients with metastatic foci of sarcoma. Cancer 1999; 86:1621-7.

23. Giannakenas C, Kalofonos HP, Apostolopoulos D, et al. Scintigraphic imaging of sarcomatous tumors with [111-In-DTPA-Phe-1]-Octreotide. Oncology 2000;58:18-24.

24. Baxter KG, Rosenthal HG, Redick ML, Preston D. In-111 pentatreotide uptake in a chondrosarcoma. Clin Nuc Med 1996;21:1008-9.

25. Redding TW, Schally AV. Inhibition of growth of the transplantable rat chondrosarcoma by analogs of hypothalamic hormones. P Nat Acad Sci USA 1983;80:1078-82.

26. Reubi JC. A somatostatin analogue inhibits chondrosarcoma and insulinoma tumor growth. Acta Endocrinologica 1985;109:104-14.

27. Pinski J, Schally AV, Halmos G, Szepeshazi K, Groot K. Somatostatin analog RC-160 inhibits the growth of human osteosarcomas in nude mice. Int J Can 1996;65:870-4.

28. Albini A, Florio T, Giunciuglio D, et al. Somatostatin controls Kaposi's sarcoma tumor growth through inhibition of angiogenesis. FASEB J 1999;13:647-55.

29. Conzemius MG, Graham JC, Haynes JS, Graham CA. Effects of treatment with growth hormone and somatostatin on efficacy of diammine [1,1-cyclobutane dicarboxylato (2-)-0,0']-(SP-4-2) in athymic rats with osteosarcoma. Am J Vet Res 2000;61:646-50.

30. Reubi JC, Kappeler A, Waser B, et al. Immunohistochemical localization of somatostatin receptors sst2A in human tumors. Am J Pathol. 153: 233-45, 1998.

31. Canobbio L, Cannata D, Miglietta L et al. Somatuline (BIM 23014) and tamoxifen treatment of postmenopausal breast cancer patients: clinical activity and effect on insulin-like growth factor-I (IGF-I) levels. Anticancer Res, 15:2687-2690, 1995.

32. Krennings EP, Kweekkeboom DJ, Bakker WH, et al. Somatostatin receptor scintigraphy with octreotide: the Rotterdam experience with more than 1000 patients. Eur J Nucl Med 1993;20:716-31.